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Classification of Peste des Petits Ruminants Virus as the Fourth Member of the Genus *Morbillivirus*

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Summary. Peste des petits ruminants (PPR) is a virus disease of sheep and goats in West Africa. When first described, the virus was considered a variant of rinderpest virus. The biological and physicochemical characteristics of the virus indicate that it is closely related to measles, rinderpest and canine distemper viruses. These three viruses form the genus *Morbillivirus* of the Paramyxoviridae. PPR virus is sufficiently distinct from these 3 viruses to justify considering it as the fourth member of the *Morbillivirus* genus.

Peste des petits ruminants (PPR) (synonyms: stomatitis/pneumo-enteritis complex and Kata) is an important virus disease that affects sheep, but more particularly goats, in West Africa.

The clinical disease is similar to rinderpest in cattle and was first described in detail in 1956 [1]. The early clinical signs of PPR are pyrexia, a catarrhal nasal and ocular discharge, and necrotic stomatitis; many animals subsequently develop a severe virus enteritis and pneumonia. In susceptible flocks, morbidity may be 100% and mortality >90%. In Nigeria, PPR probably causes an annual loss in excess of $ 1.5 million [2].

Different viruses have been isolated from affected sheep and goats, but the causal virus is accepted to be in the Paramyxoviridae family and similar in its biological characteristics to measles, canine distemper and rinderpest viruses [2–5]. Measles, canine distemper and rinderpest viruses form the *Morbillivirus* genus [6], and varying degrees of cross-protection *in vivo* and serological relationship *in vitro* exist among them [7].

Mornet et al. [1] reported in 1956 that PPR virus was not pathogenic for cattle, conferred immunity against rinderpest, and could be

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neutralized by rinderpest antiserum. Since rinderpest virus – although principally a pathogen of cattle – can produce disease in sheep and goats [8–11], they concluded that PPR virus was a variant of rinderpest virus that was adapted to small ruminants and had lost virulence for cattle. However, more recent work, details of which were published while the study reported in this paper was in progress, indicates that PPR and rinderpest viruses can be differentiated by cross-neutralization *in vitro* [2]. To our knowledge no published information is available on the relationship of PPR virus to measles and distemper viruses.

It is now important to establish the relationship of PPR virus to the other morbilliviruses, especially rinderpest, for two reasons. First, the virtual eradication of rinderpest from cattle in West Africa by the international use of vaccine (Joint Project 15 of OAU/STRC [12, 13]) raises the question whether PPR virus infection of small ruminants represents a reservoir of rinderpest infection for cattle should vaccination be discontinued. Second, a vaccine is required to control PPR in West Africa, as small ruminants are an important source of animal protein in areas where cattle cannot be raised easily because of trypanosomiasis.

This paper reports studies on the relationship of PPR virus to measles, canine distemper and rinderpest viruses and proposes the classification of PPR as the fourth member of the *Morbillivirus* genus.

Materials and Methods

Viruses

The virulent strains of virus used in these studies were PPR Nig 75/1 (passage 5 in lamb kidney cell monolayers) and rinderpest RBT/1 (passage 8 in calf kidney monolayers) [14]. PPR Nig 75/1 is serologically identical with the prototype isolate PPR Senegal [3, 14a]. The vaccine strains were rinderpest RBOK [15], canine distemper Onderstepoort [16,17], and measles Schwarz [18]. The latter two were donated by Evans Biologicals Ltd. (Speke, Liverpool, UK).

Biological and Physicochemical Characterization

The biological and physicochemical characteristics of PPR virus that are relevant to classification were determined as follows. The stained CPE in tissue culture was examined with hematoxylin and eosin, acridine orange and Feulgen stains. The nucleic acid of the genome was determined by use of bromodeoxyuridine (100 μg/ml of cell culture medium) [19], including in the test infectious bovine rhinotracheitis virus and bluetongue virus as known DNA and RNA viruses, respectively. PPR virus was assayed in lamb kidney cell monolayers, infectious bovine rhinotracheitis virus in calf kidney cell monolayers, and bluetongue virus in BHK21 Cl. 13 cell monolayers. The morphology of the virus was studied in the electron microscope by negative staining of the pellet obtained from ultracentrifugation of supernatant fluids from infected cultures; the sensitivity of the virus to ether was investigated as described by *Andrewes and Horstmann* [20].

Serological Comparisons

To investigate whether PPR and rinderpest viruses possess a common immunodiffusion antigen, suspensions of lymph nodes from cases of PPR and rinderpest were tested against rabbit serum hyperimmune to rinderpest virus by the technique described by *White et al.* [21].

For the serological comparison by cross-neutralization of PPR, rinderpest, measles, and canine distemper viruses, PPR Nig 75/1 and the virulent rinderpest isolate RBT/1 were assayed in lamb kidney and calf kidney cell monolayers, respectively; the rinderpest, measles and canine distemper vaccine strains were assayed in Vero cells. A constant virus varying serum neutralization test was used incorporating 2.0 ± 0.5 $\log_{10}$ tissue culture infective doses as the challenge virus against doubling dilutions of antiserum. The viruses were incubated with the antisera for 1 h at 37° before inoculation to tube cultures. Inoculated cells were rolled at 37°. Antisera were collected from the host species which had been either infected or vaccinated at least 21 days previously. PPR virus was also tested against antisera to bovine respiratory syncytial virus [22], bovine parainfluenza 3 virus [23], and bovine syncytial virus [24]. Antisera to measles and canine

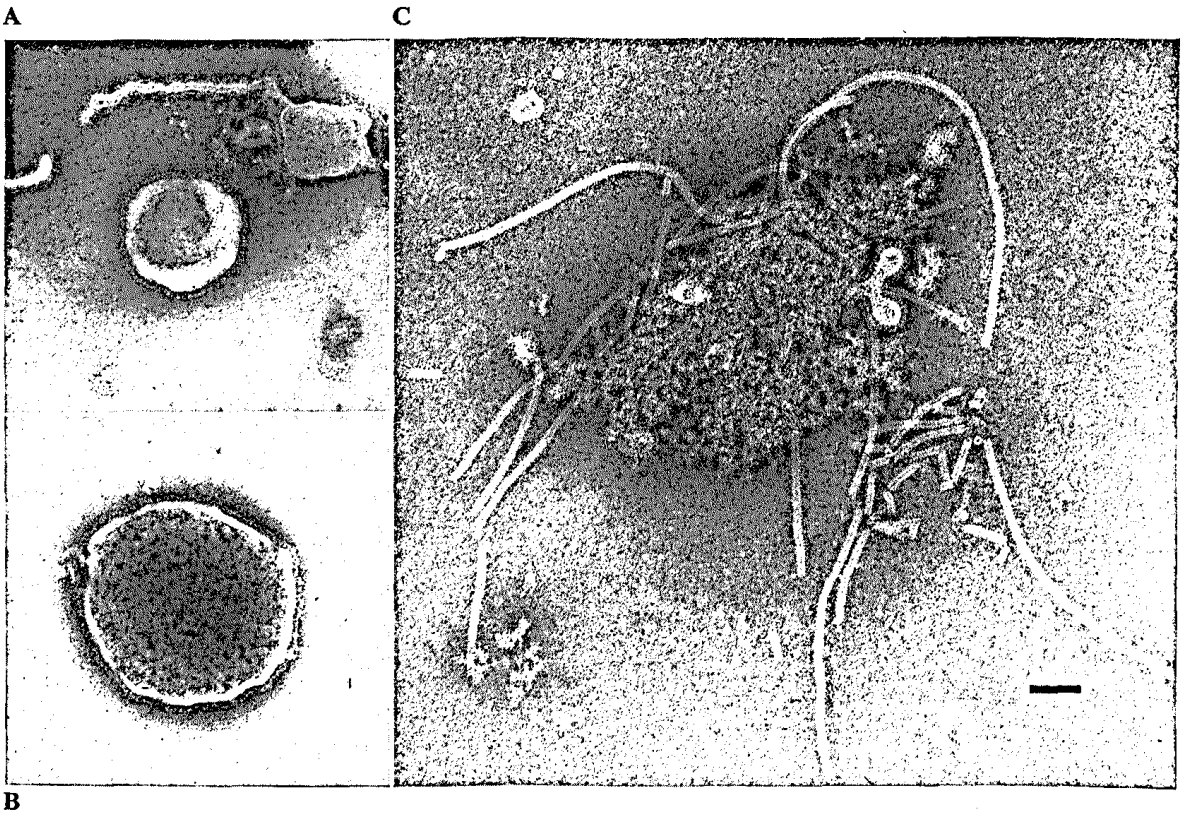

Fig. 1. Electron micrographs of PPR virus stained with 2% phosphotungstic acid, pH 7.1, and reproduced at the same magnification throughout. Bar = 100 nm. As with other morbilliviruses, PPR virus is pleomorphic and consists of an outer envelope surrounding a coiled nucleocapsid. There is considerable variation in particle size. **A** The small particle in the center of the photomicrograph is intact and not penetrated by stain; a second disrupted virus particle is seen at the top of the print. Both particles have surface projections on the envelope (surface projections: length 15.8 nm, period 5.6 nm, and width 2.8 nm). **B** A large particle penetrated by stain. **C** A disrupted particle from which the structure of the nucleocapsid can be seen (period 6.7 nm, width 18.6 nm). All dimensions are based on approximately 25 measurements with standard calibration using a Philips 301 electron microscope.

distemper viruses were donated by Dr. *J. Prydie* (Wellcome Laboratories, Beckenham, Kent, UK) and to bovine respiratory syncytial virus and bovine syncytial virus by Dr. *G.N. Wood* and Dr. *E.J. Stott* (Institute for Research on Animal Diseases, Compton, Newbury, Berks., UK).

Animal Experiments

The design of the experiments for the study of cross-protection and pathogenicity in cattle, sheep and goats is outlined in 'Results' (table III). Some of the cattle were Jersey since this breed is considered particularly susceptible to rinderpest and, by inference, the most likely to be susceptible to PPR virus should this virus prove virulent for cattle. The sheep were Dorset Horn and the goats mixed British breeds. Animals in experiment No. 1 and 2 that had been inoculated with PPR or rinderpest viruses were monitored daily from 0 to 12 days for clinical disease, pyrexia, viremia, virus excretion from the nose, mouth, urinogenital and alimentary tracts, and tested for serum antibody at 0, 7, 14 and 21 days. The sheep and goats in experiment No. 3 were examined daily for clinical disease and pyrexia. They were sampled for excretion of PPR virus 7 days after challenge. Each group was housed separately in loose boxes designed to prevent escape of infectious virus.

Results

Biological and Physicochemical Characteristics

CPE was produced by PPR virus in a variety of cell cultures, including Vero cells, but sheep and goat kidney cell cultures were considered the most sensitive for isolation and assay of the virus. In sheep and goat cell monolayers, the CPE consisted of large 'clock-faced' syncytia, the nuclei of which sometimes contained intranuclear Cowdry type A inclusions. CPE usually developed between 6 and 15 days after inoculation, the onset being dependent upon the MOI. Cells inoculated in suspension showed CPE more rapidly. The titer of virus grown in sheep and goat kidney cell monolayers rarely exceeded 6.0 $\log_{10}$ TCD_{50}/ml.

The morphology of PPR virus as seen by negative-staining electron microscopy was typical of a paramyxovirus (fig. 1).

Examination of infected cells stained with acridine orange and Feulgen reagents indicated that the genome of PPR virus was RNA. The pyrimidine analogue bromodeoxyuridine did not suppress the replication of the virus, thus providing further evidence that the nucleic acid of PPR virus was RNA. PPR virus was found to be ether-sensitive.

The above biological and physicochemical characteristics of PPR virus are summarized in table I. For comparison, data are included for other Paramyxoviridae and for viruses unrelated to PPR virus that are associated with similar diseases in sheep and goats.

Serological Relationships

PPR and rinderpest viruses were found to share a common antigen when examined by immunodiffusion, but spur formation was observed at the junction of the two lines of precipitate indicating an incomplete antigenic relationship between the two viruses.

Relationships were shown between PPR

Table I. Biological and physicochemical characteristics of PPR virus compared with those of other Paramyxoviridae and unrelated viruses associated with similar infections of sheep and goats

Virus	Morphology	Resistance to lipid solvents	Type of nucleic acid in genome	CPE in tissue culture		
				syncytia	intra-cytoplasmic inclusions	intra-nuclear inclusions
PPR	pleomorphic	sensitive	RNA	+[1]	+	+
Rinderpest	pleomorphic	sensitive	RNA	+	+	+
Canine distemper	pleomorphic	sensitive	RNA	+	+	+
Measles	pleomorphic	sensitive	RNA	+	+	+
Other viruses associated with sheep and goat infections						
Parainfluenza 3	pleomorphic	sensitive	RNA	+	+	+
Respiratory syncytial	pleomorphic	sensitive	RNA	+	+	–
Bluetongue	icosahedral	resistant	RNA	–	–	–
Foot-and-mouth disease	icosahedral	resistant	RNA	–	–	–
Adeno	icosahedral	resistant	DNA	–	–	+

1 + = Positive; – = negative.

Table II. Serological relationships of PPR virus (PPR Nig 75/1) with vaccine strains of canine distemper (Onderstepoort), measles (Schwarz) and rinderpest (RBOK)[1] viruses

Antiserum[2]	Virus			
	canine distemper	measles	rinder-pest	PPR
Canine distemper	8,200[3]	12	4	12
Measles	6	128	12	8
Rinderpest	32	32	256	12
PPR	24	12	4	1,536

[1] Rinderpest RBOK and RBT/1 viruses gave very similar results. Data for RBT/1 therefore are omitted.
[2] Serum from natural host.
[3] Reciprocal of 50% endpoint dilution of serum against 100 $TCID_{50}$ virus.

virus and canine distemper, measles and rinderpest viruses by cross-neutralization (table II), but the extent of antigenic homology was no greater between PPR virus and the other viruses than already exists among them [7]. PPR virus was not neutralized by antisera to bovine respiratory syncytial virus, bovine parainfluenza 3 virus, or bovine syncytial virus.

Pathogenicity and Cross-Protection

Subcutaneous inoculation of sheep and goats with PPR virus usually produced clinical disease and excretion of virus with spread of infection to in-contact sheep and goats (table III, experiment No. 1). PPR virus was not pathogenic for cattle and protected them from challenge with virulent rinderpest virus (table III, experiment No. 2). Neither replication nor excretion of PPR virus in cattle could be demonstrated, although serum antibody was produced. Transmission of infection from inoculated cattle to in-contact cattle did not occur.

Sheep and goats vaccinated with the attenuated RBOK strain of rinderpest virus did not develop clinical disease when infected with PPR virus. The Schwarz vaccine strain of measles virus did not protect sheep and goats from developing PPR (table III, experiment No. 3). A degree of cross-protection apparently exists between the viruses of canine distemper and PPR, since only 1 animal in the group vaccinated with canine distemper virus showed mild clinical lesions of PPR.

However, PPR virus replicates in sheep and goats given rinderpest, canine distemper and measles vaccines, virus being detected in nasal swabs collected from each group 7 days after challenge. To investigate whether goats and sheep that had recovered from clinical PPR could also excrete PPR virus if re-exposed to infection, the animals in the control group in the same trial were subsequently challenged 21 days after initial infection. PPR virus could not be recovered from nasal secretions collected 7 days later.

Discussion

The biological and physicochemical characteristics of PPR virus, as determined in this study, conform to those of the other members of the genus *Morbillivirus* [6] and to the earlier reports on this virus [2–4, 25, 26]. The serological studies justify the proposal that PPR virus should be considered as the fourth member of the *Morbillivirus* genus of the Paramyxoviridae.

The confirmation that PPR virus is not pathogenic for cattle and is not transmitted between cattle suggests that PPR is unlikely to interfere with the eradication of rinderpest in cattle in West Africa.

Table III. Pathogenicity and cross-protection studies of PPR virus and rinderpest, canine distemper and measles virus

Animal group	Virus inoculation and titer[1]		Clinical disease
	day 0	day 21	
Experiment No. 1			
4 goats	PPR Nig 75/1 (5.3)	–	PPR in inoculated and in contact animals
1 goat, 1 sheep in contact with goats from day 3			
4 sheep	PPR Nig 75/1 (5.3)	–	PPR in inoculated and in contact animals; disease mild in sheep
1 goat, 1 sheep in contact with sheep from day 3			
Experiment No. 2			
2 cattle	PPR Nig 75/1 (4.5)	–	no disease
4 cattle	PPR Nig 75/1	RBT/1 (6.7)	no disease
2 cattle in contact with above from day 3	–	RBT/1 (6.7)	rinderpest
4 cattle	–	RBT/1 (6.7)	rinderpest
Experiment No. 3			
4 goats, 4 sheep	RBOK vaccine (2.7)[2]	PPR Nig 75/1 (4.7)	no disease
4 goats, 4 sheep	CD vaccine (3.2)	PPR Nig 75/1 (4.7)	mild stomatitis in 1 goat only
4 goats, 4 sheep	M vaccine (3.7)	PPR Nig 75/1 (4.7)	PPR
4 goats, 4 sheep (control group)	–	PPR Nig 75/1 (4.7)	PPR

1 Virus inoculated subcutaneously over the shoulder.
Titers (in parentheses) expressed as $\log_{10}$ TCD_{50}/animal. PPR = peste des petits ruminants; RBT/1 = rinderpest bovine Tanzania; RBOK = rinderpest bovine 'O' Kabete (Kenya); CD = canine distemper Onderstepoort; M = measles Schwarz.
2 Recommended vaccine dose for host species.

This small study has shown that rinderpest vaccine protects sheep and goats from clinical PPR. Rinderpest vaccine is produced in large quantities in West Africa, and some of this vaccine already has been used empirically to protect sheep and goats from PPR. Although there has been no critical evaluation of its effectiveness, the 'Office International d'Epizooties' has endorsed its use [13]. However, the observation reported here that sheep and goats that have been vaccinated with rinderpest vaccine can develop subclinical PPR indicates that, under field conditions, animals vaccinated with rinderpest vaccine possibly may perpetuate PPR in sheep and goat populations even though they are themselves protected from developing clinical disease. Whether a specific vaccine for protection of sheep and goats against PPR is required will depend upon the results of field trials using rinderpest vaccine.

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